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Isolation and Characterization of *Sporothrix schenckii* from Clinical and Environmental Sources Associated with the Largest U.S. Epidemic of Sporotrichosis†

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The largest recorded epidemic of sporotrichosis in the United States occurred in 1988 and involved a total of 84 cases in 15 states. All cases were associated with Wisconsin-grown sphagnum moss. Twenty-one clinical isolates of *Sporothrix schenckii* and 69 environmental isolates of *Sporothrix* spp. from the epidemic were characterized and compared. The environmental isolates were recovered from 102 samples of sphagnum moss and other material by using direct plating techniques. Characteristics examined included macroscopic and microscopic morphology, conversion to a yeast phase, exoantigen reactions, and virulence in mice. On the basis of these studies, eight environmental isolates were identified as *S. schenckii*, five were identified as *Ophiostoma stenoceras*, and the remainder were identified as *Sporothrix* species. The environmental isolates of *S. schenckii* were recovered from moss samples from one Pennsylvania nursery and from three New York State Soil and Water Conservation districts, but none were recovered from moss directly from the bogs in Wisconsin.

In the late spring and early summer of 1988, cases of sporotrichosis began to appear among forestry workers who were participating in annual reforestation programs in New York and Illinois. An investigation identified a total of 84 cases from 15 states, thus constituting the largest known epidemic of sporotrichosis in the United States. The majority of the cases were exposed to material purchased from one Pennsylvania nursery, and all cases were associated with exposure to sphagnum moss that had been obtained from a single Wisconsin distributor (3).

Although it has been more than 90 years since Hektoen and Perkins (13) so thoroughly and elegantly described the disease sporotrichosis and its causative agent, *Sporothrix schenckii*, there is still difficulty in distinguishing the fungus from its closely related, non-sporotrichosis-causing relatives (21, 22). The 1988 epidemic of sporotrichosis provided both clinical and environmental isolates for mycological study.

The purpose of this article is to summarize the results of our studies involving (i) the isolation and characterization of *Sporothrix schenckii* from environmental samples obtained during the epidemiologic investigation to establish the source of the fungus and (ii) the comparison of the environmental isolates with the clinical isolates from the same epidemic.

MATERIALS AND METHODS

Background. Large commercial nurseries provide thousands of seedlings each spring to private retailers (such as garden clubs and Christmas tree farms) and to local soil and water conservation districts who participate in reforestation programs. Nurseries usually wrap the roots of the seedlings in wet sphagnum moss to protect and moisten them during shipment and distribution. Most of the moss used for this purpose is derived from bogs in Wisconsin. The moss is harvested from the bogs either manually or with heavy machinery and is dried in adjacent fields for several days. Dried moss is baled and bound with wire or sheets of plastic and then stored until shipping. Harvest may take place during all but the winter months. The nurseries usually receive large shipments of moss early in the year and store it until needed. Depending on when the moss is harvested and shipped and the volume of seedlings to be packed for a particular season, the moss can be from 4 months to 2 years postharvest when it is used.

Environmental samples. Following the outbreak, environmental samples (Table 1) were collected from the New York State Soil and Water Conservation (NYSWC) districts in seven New York counties, from six Pennsylvania nurseries (coded A through F), and from moss directly from the bogs in Wisconsin.

Samples consisted of sphagnum moss, dirt, evergreen seedlings and needles, hay, burlap from packing material, root gel (potassium propenoate-propenamide copolymer), pond water (used at one nursery to moisten the moss), and packing tape (Table 1). Samples were collected by a team of investigators from the New York State Health Department, the Pennsylvania State Health Department, and the Centers for Disease Control.

Environmental sample processing. Samples contained in sealable plastic sandwich bags (6.5 by 6 in. [ca. 16.5 by 15 cm]) were received between June and August of 1988. Samples were opened and incubated in sterile stainless-steel cans for a minimum of 24 h at room temperature in the presence of moth crystals (paradichlorobenzene) for arthropod control and then processed by using a modification of the direct plating technique described by Dixon and Shadomy (8). Approximately 25 cm^3 (loose volume) of sphagnum moss was taken from at least 10 different locations within a given bag to constitute an individual sample for

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TABLE 1. Summary of environmental samples and culture results

Sample	Samples cultured				No. of isolates recovered	
	Condition	No. negative[a]	No. positive[a]	Source[b]	Virulent	Avirulent
Burlap	Dry	1		N-A		
Dirt	Dry	1	1	N-A	0	1
		1		N-E		
Dirt and grass	Wet	1		N-B		
Dirt and needles	Dry	1		N-C		
Hay	Dry	1		N-A		
Moss	Dry	4	7	N-A	1	11
		4		N-B		
		1	4	N-C	0	5
		4	1	N-D	0	2
		3	2	N-E	0	4
		2		N-E		
			4	BR[c]	2	6
		2		CH		
			2	DE[c,d]	0	3
			2	GE[d]	0	7
		3	1	HE[c,d]	0	2
		1		IL		
			2	MO[d]	1	1
			2	ON[d]	2	2
		10		WI		
Moss	Mud	1		WI	0	
Moss	Wet		1	N-A	1	2
		1		N-B		
		1	1	N-C	0	1
			1	N-D	0	1
			2	N-E	0	3
		2		WI		
Moss and dirt	Dry		1	N-A	0	1
Moss and needles	Dry		1	N-A	1	1
Moss and wood	Dry		1	N-A	0	2
Needles	Dry	1		N-F		
Packing tape	Dry	1		N-E		
Seedlings	Dry		2	IL	0	5
Water		8	1	N-A	0	1
		2		N-D		
		1		N-E		
Water plus root gel		2		N-A		
Wood	Dry	3		N-A		
Total		63	39		8	61

[a] For *Sporothrix* or *Ophiostoma* sp.

[b] Abbreviations: N-A through N-F, Pennsylvania nurseries A through F, respectively; IL, Illinois; WI, Wisconsin. Abbreviations for New York State County Soil and Water Conservation Extension Service in the following counties: BR, Broome; CH, Chenango; DE, Delaware; GE, Genesee; HE, Herkimer; MO, Montgomery; ON, Onondaga.

[c] Moss and seedlings from nursery A.

[d] Moss and seedlings from nursery B.

processing. For tree seedlings, samples consisted of cuttings of needles. Each 25-cm^3 sample was deposited in a sterile, cotton-plugged, 250-ml glass Erlenmeyer flask to which was added 90 to 100 ml of antibacterial antibiotics in sterile water (5,000 U of penicillin G and 1 mg of streptomycin per ml). The flasks were agitated intermittently over a 1-h period at room temperature. At 3 to 5 min after the last agitation, 1-ml volumes of suspension were removed and diluted 1:10 and 1:100 in sterile water. Volumes (0.2 ml) of undiluted, 1:10-diluted, and 1:100-diluted suspensions were then spread with sterile swabs onto separate Mycosel agar (Myco; BBL, Cockeysville, Md.) plates (100 by 15 mm). Liquefied root dip and pond water were plated directly in 0.2-ml volumes. Plates were incubated at 30°C and monitored for growth.

Initial evaluation of fungal growth. Plates were examined at regular intervals, and squashed or teased preparations were made of representative colonies from each sample for microscopic examination. Colony counts were made after 5 to 10 days of incubation. Representative colonies suspected of being *S. schenckii* were subcultured onto modified Sabouraud dextrose agar (Difco, Detroit, Mich.) supplemented with 20 U of penicillin per ml and 40 μg of streptomycin per ml (MSDA+).

Fluorescence microscopy of environmental samples. The first four samples of sphagnum moss submitted (from the NYSWC districts) were also used to compare direct plating with direct microscopic examination by fluorescent-antibody technique and calcofluor white (Uvitex; Ciba Geigy, Basel, Switzerland) screening as a means of evaluating the presence of *S. schenckii*. After the samples were processed by using the above aqueous extraction procedure, suspensions from the extraction flasks containing samples from the same source were pooled, filtered through sterile gauze, and centrifuged at 200 × *g* for 10 min. One loopful of sediment from each pooled sample was then streaked in duplicate on Myco plates for incubation at 30°C and monitored as described above. The pellets were frozen, stored, and later thawed for direct microscopic examination. Smears were made from each of the sample precipitates and screened by using a fluorescein-labeled rabbit anti-*S. schenckii* antibody (10). Smears were made as described above and stained with calcofluor white for examination by UV fluorescence microscopy.

Characterization of the isolates on the basis of morphology. Isolates presumptively identified as *Sporothrix* sp. were streaked for isolation on Sabouraud dextrose agar (S+; Difco) plates supplemented with 40 μg of gentamicin per ml (Schering Corp. Kenilworth, N.J.) and 25 μg of chloramphenicol per ml (Sigma Chemical Co., St. Louis, Mo.) to check for purity and to compare morphology. Plates were incubated at 30°C for 7 days, and the resulting colonial morphology was evaluated. Isolated colonies were subcultured to MSDA+, and the resulting growth was used to inoculate potato dextrose agar (PDA; Difco) slide cultures, which were incubated at 30°C and mounted after 14 days. Each isolate was also inoculated on slants of five media: S+, Myco, PDA, malt (Difco), and cornmeal (CM) (supplemented with 1% Tween 80; Difco). The cultures were incubated at 27°C and observed at 7, 14, and 30 days and again at 60 days for colony color and the presence of perithecia. Microscopic morphology was evaluated by using mounts from the PDA slide cultures. Measurements of microscopic structures were made by use of a calibrated ocular micrometer. All measurements are based upon a minimum of 10 conidia.

Clinical isolates of *S. schenckii*. The sources and strain designations of the 21 clinical isolates from the epidemic are shown in Table 2.

Evaluation of growth capability at various temperatures. Mature MSDA+ slant cultures were used to prepare inoculum by flooding the surface with sterile water and gently rubbing the surface growth with a pipette. The resulting suspensions were used to inoculate slants (0.1 ml per slant) of MSDA+ and cystine heart hemoglobin agar (Difco) supplemented with 20 U of penicillin per ml and 40 μg of streptomycin per ml (CHHA+). CHHA+ slants of each isolate were incubated at 35 and 37°C; MSDA+ slants of each isolate were incubated at 30 and 37°C. Incubation at

TABLE 2. Clinical isolates from 21 patients[a] from the 1988 epidemic of sporotrichosis

Accession no.[b]	Anatomical site[c]	Specimen[d]	State
M673-88	L midfinger	RC	Ill.
M675-88	L hand	RC	Ill.
M676-88	R index finger	RC	Ill.
M677-88	Finger	RC	Ill.
M923-88	Arm (multiple knots)	RC	Mich.
M516-88, M549-88	L thumb	FA, RC	N.Y.
M527-88	R hand	PS	N.Y.
M535-88	Arm	PS	N.Y.
M537-88	Index finger	RC	N.Y.
M546-88	L hand	PS	N.Y.
M547-88, M554-88	Hand	RC, RC	N.Y.
M576-88	R index finger	RC	N.Y.
M611-88	R wrist (multiple)	PS	N.Y.
M619-88	R forearm (multiple)	PS	N.Y.
M622-88	Unknown	FA	N.Y.
M639-88	R index finger	RC	N.Y.
M644-88, M647-88	R forearm	FA, RC	N.Y.
M665-88	L upper arm (multiple)	RC	N.Y.
M817-88	Hand	RC	N.Y.
M708-88	L forearm	RC	Pa.
M808-88	L arm (multiple)	PS	Pa.

[a] All patients had contact with material purchased from Pennsylvania nursery A.

[b] Specimens (New York State Department of Health Mycology Laboratories accession numbers) from the same patient are listed together, separated by a comma.

[c] Single lesion except where noted. L, left; R, right.

[d] Specimen submitted: RC, reference culture; FA, histopathologic section for specific fluorescent-antibody identification; PS, primary specimen.

37°C was in the presence of 5% CO_2. Macroscopic growth was evaluated after 21 days as present or absent. The remainders of the suspensions were deposited in a sterile water culture collection (16).

Evaluation of conversion to a yeast phase. After initial studies were conducted with several media and incubation conditions, the following procedure was adopted for testing all isolates for conversion to the yeast phase. Volumes (0.5 ml) of brain heart infusion broth containing 0.1% agar (BHIB+) were added to slants of each isolate with Pasteur pipettes. The surface growth was washed gently to prepare a suspension of conidia for inoculation into screw-cap tubes (20 by 150 mm) containing 10 ml of BHIB+ (19). Depending on the maximum temperature of growth of each isolate, the inoculated broths were incubated at 35 or 37°C in the presence of 5% CO_2. Each isolate was evaluated at day 4 and day 12 by making mounts for phase-contrast microscopy. Conversion was considered to have occurred if ≥10% of the cells in a given high-power field were budding, with visible communication of cytoplasm connecting mother and daughter cells. All isolates showing <10% conversion were serially transferred to fresh tubes of BHIB+ and incubated under the same conditions. Mounts were again made at 4 and 12 days for evaluation of growth.

Exoantigen test. The microimmunodiffusion procedure used was that of Kaufman and Standard (15). The control antigen was prepared from a yeast-phase culture of a clinical isolate (New York State Mycology Culture Collection M195) as described by Palmer et al. (17). Control rabbit antiserum was prepared against heat-killed antigen. Twenty-one clinical isolates from the epidemic and 69 environmental strains representing each of six morphological groups were tested. Also tested were four isolates of *Sporothrix cyanescens* (M727-87, M70-88, M756-87, and M883-87), six of *Chaetomium* sp. (141B, M267-84, M778-84, M916-84, M1543-85, and M658-56), one of *Ophiostoma stenoceras* (M674-88), four of *Acremonium* spp. (environmental isolate 25 and culture collection isolates 339a, 435a, and 445a), and control antigen and antisera for *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Coccidioides immitis* (Scott Laboratories, Inc., Fiskeville, R.I.). Cultures were grown on S+ or MSDA+ slants at 27°C for 2 weeks and extracted, concentrated 25-fold, and tested.

Growth response to thiamine. Asparagine-glucose-salts medium contained (per liter of water) 1.0 g of *L*-asparagine, 10.0 g of glucose, 3.3 g of Na_2HPO_4, 1.1 g of KH_2PO_4, 0.25 g of $MgSO_4 \cdot 7H_2O$, 0.25 g of NaCl, and 15 g of agar with or without 5.0 mg of thiamine (14). Four representatives of each of the six groups of environmental isolates and 21 clinical isolates were studied. Pinpoint inoculum was transferred to medium with and without thiamine. Each isolate was tested in duplicate. Cultures were incubated at 30°C and examined weekly for 4 weeks.

Mating studies. Ten environmental isolates (two of each group except group II, the perithecia producers) and five clinical isolates of *S. schenckii* were selected to determine if any of the nonperithecial producers could be mated in pairs to produce a sexual stage. All isolates were inoculated on PDA slants and grown for 7 days at 30°C. Each slant was flooded with 5 ml of saline, and the colony was rubbed gently with the pipette to prepare conidial suspensions. The 10 environmental isolates were crossed in 45 different combinations. The five clinical isolates were crossed in all 50 possible combinations with the environmental isolates. For each cross, 0.2 ml of each of the two suspensions was pipetted into a sterile cork-stoppered tube (16 by 75 mm); 0.2 ml of this mixture was pipetted onto a petri plate (100 by 15 mm) of CM plus 1% Tween 80 and spread evenly over the entire surface of the plate with a sterile cotton swab dipped in sterile saline. When the plates were dry, they were inverted and incubated at 27°C for 30 days. Plates were evaluated macroscopically and with a dissecting microscope for the presence of fructifications.

RESULTS

A total of 102 environmental samples associated with the outbreak of sporotrichosis were processed by using the aqueous extraction and direct plating techniques (Table 1). Sixty-nine isolates with a presumptive identification of *Sporothrix* sp. were recovered from 39 of the samples. Most of the isolates were from sphagnum moss, but seedlings and water samples associated with moss were also positive. The presumptive identification was based upon microscopic observation of ovoid to clavate conidia borne in sympodial arrangements on lateral conidiophores.

The primary cultures of *Sporothrix* sp. on Myco were of three types: spreading, white, waxy, and glabrous (39 isolates); spreading, white, and waxy with aerial hyphae (20 isolates); and restricted, waxy, and brown to black) (10 isolates) (Table 3). Because of the range of morphology represented by these isolates, it was necessary to screen microscopically virtually all representative colony types that were not brightly pigmented. Three of the isolates that were originally brown to black on Myco subcultured as nondematiaceous molds. Conversely, three of the isolates that grew as white, waxy, spreading colonies on primary Myco plates developed dark-brown pigment with repeated subculture.

The environmental isolates of *Sporothrix* sp. comprised an

TABLE 3. Summary of environmental isolates of *Sporothrix* sp. with respect to primary isolation type versus final group

Primary Mycosel culture type	No. (%) of isolates determined to be in group[a]:						
	I	II	III	IV	V	VI	All
1[b]	1 (3)	5 (13)	10 (26)	15 (38)	5 (13)	3 (7)	39 (56.5)
2[c]	2 (10)	0	1 (5)	5 (25)	8 (40)	4 (20)	20 (29.0)
3[d]	7 (70)	0	1 (10)	1 (10)	0	1 (10)	10 (14.5)
Total	10 (14)	5 (7)	12 (18)	21 (30)	13 (19)	8 (12)	69

[a] Environmental groups are defined in Table 4.
[b] Spreading, white, waxy colony.
[c] Spreading, white, waxy with aerial hyphae on most of colony.
[d] Restricted, waxy, brown to black colony.

average of 18 to 38% of the total fungal growth on a given plate at a given dilution. Other fungal growth commonly encountered included *Penicillium* spp., *Acremonium* spp., and zygomycetes. However, the dilutions plated were appropriate to give sufficient isolated colonies of *Sporothrix* spp. if selected by day 5 for further evaluation.

Initial morphological characterization of the environmental isolates of *Sporothrix* spp. on S+ and PDA resulted in the recognition of six morphological groups (Table 4). Group I produced dematiaceous colonies on PDA as well as on CM, Myco, and malt, but not on S+; none of the other groups produced dematiaceous colonies. The production of dematiaceous colonies by group I isolates on PDA, CM, Myco, and malt slants corresponded to the production of dematiaceous conidia in PDA slide cultures. Groups II to VI lacked dark-brown or black pigmentation on any of the media tested. Group II produced perithecia in 14-day-old PDA slide cultures; perithecia were also produced by some of these isolates on CM in less than 1 week and on malt after 1 month. Groups III, IV, V, and VI were similar in appearance. They were differentiated on S+ on the basis of glabrous, waxy colonies with or without the production of diffusible pigment (III versus IV, respectively), and those which produced coarse, "ground-glass" colonies with or without aerial hyphae (V versus VI, respectively).

The 21 clinical isolates associated with the epidemic were strikingly similar in appearance to each other and to the group I environmental isolates: all were darkly pigmented on CM, PDA, Myco, and malt, and gray to taupe on S+. They produced dematiaceous conidia in PDA slide culture but did not produce perithecia.

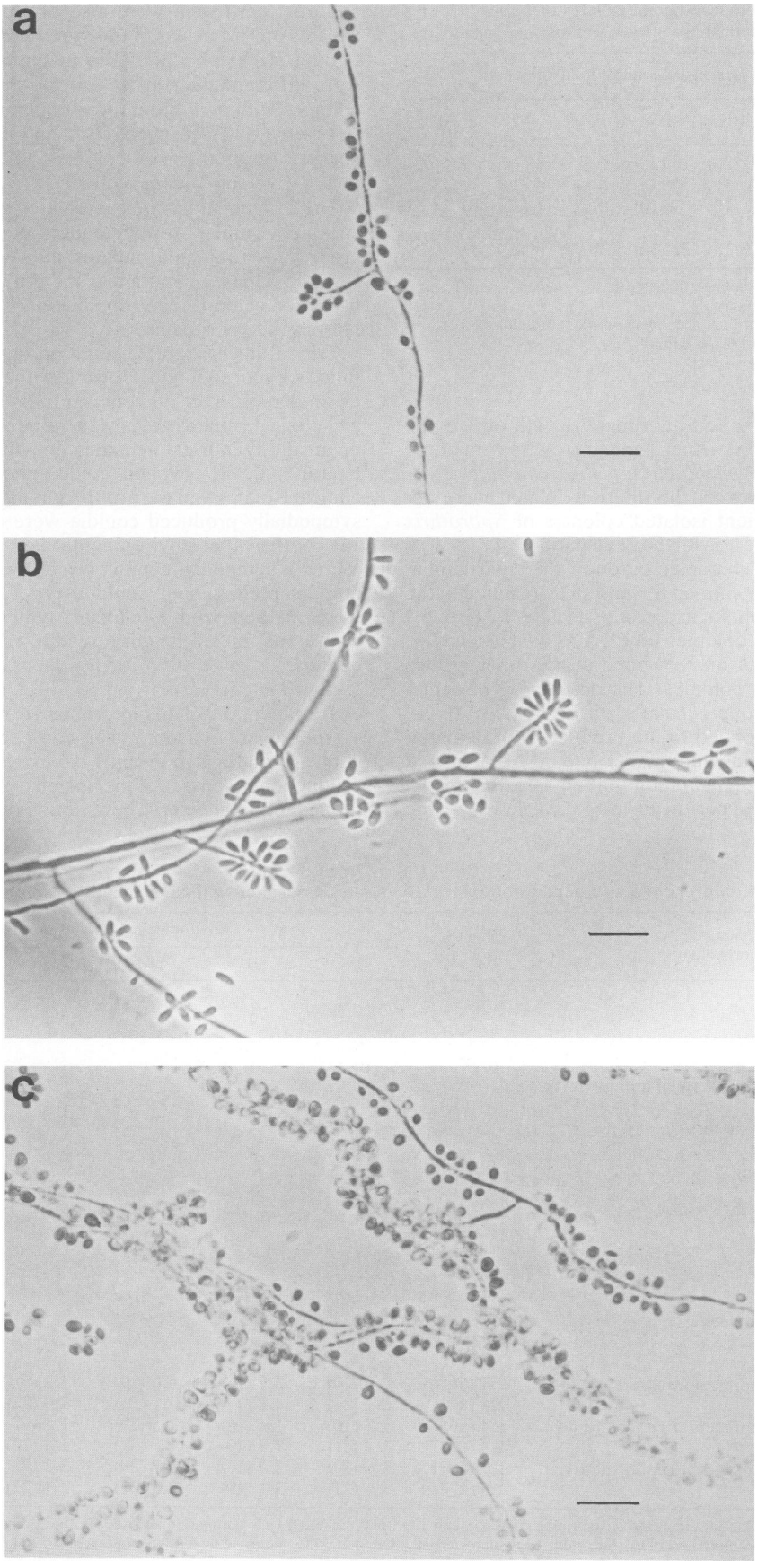
Critical microscopic evaluation of the isolates revealed a range of morphology. However, there was one consistent characteristic seen in representatives of all environmental and clinical isolates. This was the production of oval conidia on small, cylindrical denticles in sympodial arrangement on lateral, apically swollen conidiophores (Table 4). In the clinical isolates and the group I environmental isolates, these sympodially produced conidia were consistently oval (Fig. 1a). In the other environmental isolates, groups II through VI, oval sympodial conidia were always seen but they were not the predominant conidial type. Rather, these isolates were characterized by clavate, sympodial conidia (Fig. 1b). Thus, the mean length-to-width ratio of the sympodial conidia was generally ≤ 1.5 for the clinical isolates and group I environmental isolates and >1.5 (usually >2.0) for the environmental isolates in groups II through VI (Table 4).

Another microscopic characteristic common to both the clinical isolates and group I environmental isolates was the production of masses of melanized oval conidia directly from the main hyphal axis. These conidia were usually formed in

TABLE 4. Morphological groupings of *Sporothrix* isolates associated with an epidemic of sporotrichosis

Macroscopic or microscopic morphology[a]	Clinical isolates	Environmental isolates of group no.:					
		I	II	III	IV	V	VI
Macroscopic							
Color							
Grey or taupe	+	+	−	−	−	−	−
Ivory to beige	−	−	+	+	−	+	+
Tan to brown with brown diffusible pigment	−	−	−	−	+	−	−
Appearance							
Small, slightly raised, smooth	+	+	−	−	−	−	−
Finely wrinkled	−	−	+	+	+	−	−
Flat, smooth, spreading	−	−	−	−	−	+	+
White aerial hyphae	−	−	−	−	−	−	+
Texture							
Glabrous, waxy	−	−	+	+	+	−	−
Dull granular	+	+	−	−	−	−	−
Coarse "ground glass"	−	−	−	−	−	+	+
Microscopic							
Dematiaceous conidia in sleeves	+	+	−	−	−	−	−
Perithecia	−	−	+	−	−	−	−
Conidial dimensions[b]							
Mean length in μm (representative isolate no.)	2.4 (665)	2.0 (10)	3.5 (16)	4.0 (29)	3.2 (9)	4.2 (11)	3.6 (28)
	2.6 (673)	2.1 (21)	4.3 (49)	4.1 (31)	4.0 (22)	3.4 (27)	3.8 (38)
Mean width in μm (representative isolate no.)	1.4 (665)	1.3 (10)	1.4 (16)	1.5 (29)	1.5 (9)	1.4 (11)	1.4 (28)
	1.4 (673)	1.4 (21)	1.4 (49)	1.5 (31)	1.3 (22)	1.2 (27)	1.5 (38)
Mean ratio of length to width (representative isolate no.)	1.7 (665)	1.6 (10)	2.5 (16)	2.7 (29)	2.2 (9)	3.1 (11)	2.6 (28)
	1.8 (673)	1.6 (21)	3.1 (49)	2.8 (31)	2.9 (22)	2.7 (27)	2.6 (38)

[a] Isolates were streaked on Sabouraud dextrose agar plus penicillin and streptomycin (SAB+) to compare macroscopic colony morphology at 7 days. Colonies from these plates were then inoculated on PDA slide cultures, which were incubated at 30°C, mounted at 14 days, and used to evaluate microscopic morphology.
[b] Attached sympodial conidia.


a
b
c

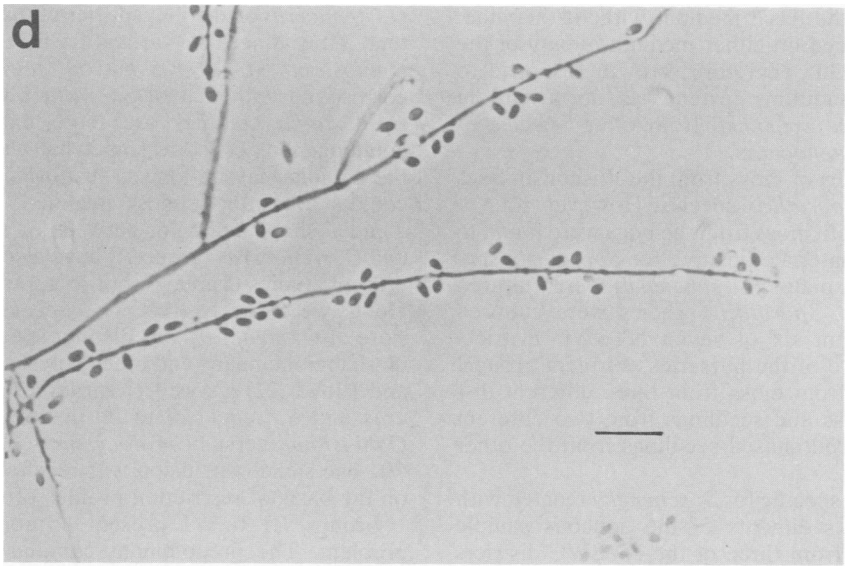


FIG. 1. Phase-contrast photomicrographs of *S. schenckii* (a and c) and of the *Sporothrix* anamorph of *O. stenoceras* (b and d; strain 16). Bar, 10 μm. (a) Oval conidia borne sympodially on a lateral conidiophore and solitary on the main hyphal axis; clinical isolate M665-88. (b) Clavate, sympodial conidia. (c) Sleeves of lateral conidia from an environmental group I isolate. (d) Solitary, lateral conidia.

such abundance as to cover the hyphae in a cylindrical layer referred to as a sleeve (Fig. 1c). This arrangement was not seen in the other isolates. However, solitary, oval, lateral nonpigmented conidia borne on short, lateral conidiophores were seen in most of the isolates (Fig. 1d).

All but one of the 69 environmental isolates of *Sporothrix* sp. in this study grew at 35°C. Only 29 of the 69 isolates grew at 37°C. These isolates included representatives of each group except group II. All of the clinical isolates grew at 37°C.

At 37°C with 5% CO_2, growth on CHHA+ and BHI agar was primarily mycelial or solitary ovoid to clavate structures indistinguishable from conidia. In BHI broth with 0.1% agar, all of the clinical isolates and 20 of the 29 environmental isolates capable of growth at 37°C converted to a yeast phase characterized by multiple elongate buds emanating from either ovoid or elongate cells. For some isolates, serial subcultures were required to initiate this budding process.

The isolates that did not grow at 37°C were tested for yeast conversion in BHI broth plus 0.1% agar incubated at 35°C with 5% CO_2. Twenty-eight of 39 isolates converted to a yeast phase. None of the group II isolates (*O. stenoceras*) converted to a yeast phase; however, these isolates did not grow well in BHIB+ at this temperature (Table 5).

The exoantigen test identified all 21 clinical isolates and all 69 environmental isolates as *S. schenckii*. Two or three precipitin bands of identity were obtained. All of the other species tested in the exoantigen study were negative. There was no correlation between number of precipitin bands and the source of the isolates.

Six representative clinical isolates and all of the environmental isolates were studied in a mouse model of disseminated sporotrichosis following intravenous injection of conidia (8a). All clinical isolates and all isolates from environmental group I with the ability to grow at 37°C produced fatal infection in mice. Two environmental isolates from group I unable to grow at 37°C did not produce fatal infection in mice. None of the isolates from groups II through VI produced fatal infection nor obvious signs of disseminated disease (8a).

Five environmental isolates (group II) were found to produce spherical black perithecia (50 to 200 μm in diameter) that were macroscopically visible on CM slants after 4 to 7 days of incubation at 30°C. Perithecia were detectable in PDA slide cultures at 14 days. Crescent-shaped ascospores 2.9 to 3.4 by 1.1 to 1.6 μm were produced in the perithecial cavity and extruded through necks up to 1,000 μm long to collect in a drop of clear exudate at the ostiole. These isolates were identified as *O. stenoceras* (Robak) Melin & Nannf. Abortive perithecial production also occurred with certain environmental isolates in groups III to VI. No fruiting structures resulted from any of the 45 crosses of environmental against environmental isolates nor from any of the 50 crosses of clinical against environmental isolates.

All of the clinical isolates and all except one of the environmental isolates tested (isolate 30, group I) grew to larger-colony diameters on asparagine-glucose-salts medium

TABLE 5. Growth temperature and yeast conversion of environmental and clinical *Sporothrix* isolates

Isolate group	Total no. of isolates	No. (%) of isolates showing conversion[a]/total no. at:		Total no. (%) of isolates showing yeast conversion[b]
		37°C	35°C	
Environmental				
I	10	8/8	2/2	10/10 (100)
II	5	0/0	0/4[c]	0/5 (0)
III	12	2/3	7/9	9/12 (75)
IV	21	1/3	16/18	17/21 (81)
V	13	5/9	1/4	6/13 (46)
VI	8	4/6	2/2	6/8 (75)
Total	69	20/29 (69.0)	28/39 (71.8)	
Clinical	21	21/21 (100)		21/21 (100)

[a] Isolates that did not grow at 37°C and isolates that grew but did not convert at 37°C were tested at 35°C.

[b] Data are expressed as the number of isolates converting to the total number tested.

[c] One isolate did not grow at 35°C.

with thiamine than on the basal medium without thiamine. Pigment was not produced on either medium by any of the isolates except isolate 30. Therefore, growth response to thiamine under the conditions tested was not useful in separating isolates of *S. schenckii* from other species of *Sporothrix* or from *O. stenoceras*.

None of the 13 samples of moss from the Wisconsin bogs were positive for any *Sporothrix* species. However, four of six nurseries supplied with moss from the bogs were found to have moss and other materials positive for *Sporothrix* species. Virulent group I isolates (*S. schenckii*) were cultured from only one nursery. *Sporothrix* species were cultured from moss samples from six of seven NYSWC districts supplied by one or more of the nurseries. Virulent group I isolates were cultured from moss from three different districts that obtained moss and seedlings from two different nurseries. One nursery purchased seedlings from the other before distribution.

Fluorescent antibody specific for *S. schenckii* reacted with smears prepared from sediments of the aqueous sample extraction suspensions from three of the NYSWC districts that were positive for either *S. schenckii* or *Sporothrix* species by culture but did not react with smears of sediment from the District that was culture negative. Calcofluor white revealed numerous structures consistent with a variety of fungal species, including clavate conidia similar to the fluorescent antibody-positive structures.

DISCUSSION

These studies involve the largest documented outbreak of sporotrichosis in the United States. With 21 clinical and 69 environmental isolates of fungi associated with the epidemic, there are numerous questions that can be addressed, including the following. (i) What constitutes a typical clinical isolate of *S. schenckii*? (ii) What constitutes a typical environmental isolate of *S. schenckii*? (iii) What is the relationship between clinical and environmental isolates? (iv) What is the relationship between *O. stenoceras* and *S. schenckii*? (v) Why were no isolates of *S. schenckii* recovered from sphagnum moss at the bogs whereas moss shipped from the same bogs to various nurseries was positive for *S. schenckii*? (vi) What do environmental isolates of *Sporothrix* sp. look like upon primary culture relative to subsequent culture?

The first question is readily answered by the data in this study. The clinical isolates conformed to published descriptions of *S. schenckii* Hektoen and Perkins (13). They produced oval conidia in sympodial arrangements on lateral conidiophores. They produced sleeves of dematiaceous conidia around the main hyphal axis when grown on the appropriate media (PDA, CM, malt, and Myco). The colony pigment seen in isolates on these media appeared to be related to the production of the dematiaceous conidia. The isolates grew at 37°C and converted to a yeast phase. The isolates were positive in an exoantigen test with antiserum raised against a control isolate of *S. schenckii*. Additionally, the isolates produced fatal disease in a mouse model of sporotrichosis.

The environmental isolates in group I are identical to the clinical isolates in every aspect studied and also with the original description of *S. schenckii* by Hektoen and Perkins; therefore, we can classify them as *S. schenckii* Hektoen and Perkins. De Hoog (6) published a long list of synonyms for *S. schenckii* on the basis of its morphology and treated them all under the ascomycete name of *O. stenoceras*. Although he did not choose to separate *S. schenckii* and the anamorph of *O. stenoceras* as distinct species at that time, de Hoog stated that "the range of variability in the conidial states of *Ophiostoma stenoceras* and *O. tetropii* does not exactly correspond with that of *Sporothrix schenckii*: usually, the *Ophiostoma* colonies are more extended, floccose, the conidiogenous cells are somewhat wider and more regular, the conidia may be longer, fusiform, and finally the lateral conidia are often almost hyaline." These differences in conidial shape and color between *S. schenckii* (our group I) and *O. stenoceras* (group II) have also been confirmed in the present studies (Table 4), but, contrary to the decision of de Hoog, we chose to treat *S. schenckii* and the anamorphic state of *O. stenoceras* as distinct species. Subsequent work on rhamnomannans and other polysaccharides by Travassos and Lloyd (22) showed *Ceratocystis* (*Ophiostoma*) *stenoceras* and *S. schenckii* to be distinct. For a discussion of *Ophiostoma* versus *Ceratocystis*, see de Hoog and Scheffer (7). The same conclusion was reached by Suzuki et al. (21) on the basis of restriction profiles of mitochondrial DNA.

Groups III to VI present a more difficult taxonomic problem. The predominant conidial morphology in these isolates is clavate as in group II rather than ovoid as in the clinical isolates and in group I (*S. schenckii*). Furthermore, the members of groups III to VI as well as group II failed to produce sleeves of dematiaceous conidia and did not produce darkly pigmented colonies on permissive media such as PDA, CM, malt, or Myco, features which are characteristic of *S. schenckii*. Additionally, none of these isolates proved to be virulent in our mouse model of infection. Since groups III to VI share more biological and morphological similarities with group II than they do with group I (Table 4), they will be referred to as *Sporothrix* spp. in this study and treated as distinct from *S. schenckii*.

Although all of the isolates of *S. schenckii* produced dematiaceous colonies upon subculture to media such as Myco, PDA, CM, and malt, 3 of the 10 environmental isolates were white upon primary isolation on Myco; conversely, three isolates later classified as *Sporothrix* sp. showed brown pigmentation upon primary isolation and failed to produce dematiaceous colonies on subculture. This must be considered in future environmental sampling attempts.

Isolates of virulent *S. schenckii* were recovered from one nursery and three NYSWC districts, whereas all samples of moss tested from the Wisconsin bogs were negative. Eight previous epidemics of cutaneous sporotrichosis that were associated with sphagnum moss have been reported in the U.S. literature (1, 2, 4, 5, 9, 11, 12, 18). In seven previous studies, the moss was traced to a Wisconsin source (2, 4, 5, 9, 11, 12, 18). In those investigations where environmental samples were obtained from the Wisconsin sphagnum bogs, no *S. schenckii* was recovered (2, 4, 20). Shippee (20) also failed to culture *S. schenckii* from bogs in Wisconsin in an environmental study of possible sources of *S. schenckii* involving sphagnum moss. Shippee did, however, culture perithecia-producing isolates and white *Sporothrix* strains from sphagnum moss from the bogs.

It is possible that the fungus is introduced into the moss after it has been harvested and transported to distribution sites. Alternatively, it is possible that *S. schenckii* is present in the bogs but at concentrations too low to be detected by the sampling methods used. Furthermore, one must consider that a bog represents an enormous sample volume, and only 13 samples were evaluated from the bogs in the present study. There is also the consideration that there was a 2- to 3-year period between moss harvest and the 1988 epidemic.

Thus, our 13 samples were from a different crop of moss than that represented directly in the epidemic. Additional studies will be necessary to determine the point at which sphagnum moss becomes contaminated with *S. schenckii*.

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